TABLE II

Peptide	Sequence	Competitor[2] [μM] 50%	Relative Competitor activity	SEQ ID NO:
Melan-A_{27-35}	AAGIGILTV	60	1	2
	ALGIGILTV	1.5	40	5
	AMGIGILTV	2	30	6
	LAGIGILTV	65	1	7
	MAGIGILTV	55	1	8
Melan-A_{26-35}	EAAGIGILTV	15	4	1
	ELAGIGILTV	6.5	9	9
	EMAGIGILTV	20	3	10
	EALGIGILTV	100	0.6	11
	EAMGIGILTV	100	0.6	12
	YAAGIGILTV	4	15	13
	FAAGIGILTV	2	30	14
Influenza A matrix$_{58-66}$	GILGFVFTL	1	60	17
MAGE-$3_{168-176}$	EVDPIGHLY	>100	<0.6	21

SEQ ID NOS: 1, 5, 6, 9, 10, 13 and 14 all showed higher affinity than SEQ ID NO: 2.

EXAMPLE 4

One concern in developing MHC binding peptides is that the resulting complexes of MHC molecule and peptide be stable, preferably more stable than the peptide originally found complexed to the MHC molecule.

Substituting the N-terminal amino acid of SEQ ID NO: 2 with Leu or Met enhanced activity between 7.5 and 20 fold, while substitutions at the second position nearly abolished it, even though binding to HLA-A*-0201 was increased (Table III and Figure 3).

SEQ ID NO: 1 was better recognized than SEQ ID NO: 2, and substitution of Ala in the second position of SEQ ID NO: 1 increased recognition 30- and 600 fold, respectively. Such substitutions at position 3 reduced activity, which was expected. Substitution of position 1 resulted in an increase in recognition.

TABLE III

Peptide Sequence	TILN LAU 203		TILN LAU 132		
	[nM]	Relative activity	[nM]	Relative activity	SEQ ID NO:
AAGIGILTV	60	1	30	1	2
ALGIGILTV	>1000	<0.6	>1000	<0.03	5
AMGIGILTV	>1000	<0.6	>1000	<0.03	6
LAGIGILTV	6	10	1.5	20	7
MAGIGILTV	8	7.5	2.5	12	8
EAAGIGILTV	12	5	3	10	1
ELAGIGILTV	2	30	0.05	600	9
EMAGIGILTV	2	30	0.05	600	10
EALGIGILTV	>1000	<0.06	>1000	<0.03	11
EAMGIGILTV	>1000	<0.06	>1000	<0.03	12
YAAGIGILTV	5	20	1	30	13
FAAGIGILTV	1	60	0.05	600	14

The results obtained with CTLs are presented herein. Specifically, five independent HLA-A*0201 restricted Melan-A specific CTL clones were used, each of which is known to lyse melanoma target cells.

The CTLs recognized SEQ ID NO: 2 with varying efficiency. When Leu was used to substitute Ala at position 1, four of the five clones showed enhanced recognition, while similar substitutions at position 2 resulted in a loss of activity. Three of the five clones recognized SEQ ID NO: 1 more efficiently than SEQ ID NO: 2 but all recognized SEQ ID NO: 9 very efficiently, while recognition of SEQ ID NO: 10 resulted in decreased efficiency of recognition

to differing degrees, and SEQ ID NO: 11 resulted in reduced recognition for four of five. When SEQ ID NO: 12 was tested, it was surprising that recognition improved, because TIL recognition decreased. With respect to SEQ ID NOS: 13 and 14, there was reduced recognition by the CTLs.

It can be gathered from this that SEQ ID NOS: 7 and 9 were better recognized, consistently, than the other peptides tested, while other peptides were recognized to different degrees.

TABLE IV

Recognition of peptide analogs by Melan-A specific CTL clones

		Recognition by clone									
		M77.86		7.10		M77.80		M77.80		1.13	
SEQ ID NO:	Peptide sequence	Peptide [nM] 50%	Relative activity	Peptide [nM] 50%	Relative activity	Peptide [nM] 50%	Relative activity	Peptide [nM] 50%	Relative activity	Peptide [nM] 50%	Relative activity
2	AAGIGILTV	15	1	50	1	300	1	300	1	4000	1
5	ALGIGILTV	90	0.16	>1000	<0.015	>1000	<0.3	>1000	<0.3	>10000	<0.4
6	AMGIGILTV	>1000	<0.015	>1000	<0.015	>1000	<0.3	>1000	<0.3	>10000	<0.4
7	LAGIGILTV	0.08	187	1.5	33	150	2	0.03	10000	30	130
8	MAGIGILTV	0.6	25	15	3	200	1.5	0.5	600	80	50
1	EAAGIGILTV	0.15	100	4	12	0.06	5000	600	0.5	2000	2
9	EALGIGILTV	300	0.05	>1000	<0.015	40	7.5	>1000	<0.3	>10000	<0.4
10	EAMGIGILTV	0.5	30	1	50	0.02	15000	5	60	50	80
11	ELAGIGILTV	0.015	1000	0.5	100	0.015	20000	0.5	600	20	200
12	EMAGIGILTV	550	36	>1000	<0.015	40	7.5	>1000	<0.3	>10000	<0.4
13	YAAGIGILTV	0.015	1000	35	1.4	>1000	<0.3	1000	0.3	>10000	<0.4
14	FAAGIGILTV	0.005	3000	7	7	>1000	<0.3	>1000	<0.3	200	20

Relative antigenic activity of Melan-A derived peptides was measured as described in the legend to FIG. 4 and table III.

Additional experiments are depicted in Figure 3 which show recognition of various Melan-A peptide analogues presented by T2 cells, by TILN LAU203 and TILN LAU132. A 4-hour ^{51}Cr assay was conducted at a lymphocyte to target ration of 30:1.

The first panels of Figure 3 (top and bottom) compare SEQ ID NOS: 2, 7, 8, 5, and 6. The second set of panels (top and bottom) compare SEQ ID NOS: 1, 9, 10, 11, and 12. The third set (top and bottom) compares SEQ ID NOS: 1, 13, 14, and 4.

Note that there was barely any activity with the parental peptides in sample LAU203, while SEQ ID NO: 9 elicited a strong CTL response. This activity was also cross reactive with SEQ ID NO: 1.

The results in the following table describe experiments using the same peptides and using PBL from eight different HLA-A2 positive melanoma patents, LAU203, LAU132, LAU145, LAU86, LAU50, LAU148, LAU161, and LAU119.

TABLE V

tested on: Patient		Percentage specific lysis from cultures stimulated with peptide[a] SEQ ID NO: 2 Melan-A 27-35				SEQ ID NO: 4 Melan-A 26-35				SEQ ID NO: 5 Melan-A 26-35 A27L			
code	E/T[b]	T2	T2 + M10	Me260	Me290	T2	T2 + M10	Me290	Me260	T2	T2 + M10	Me290	Me260
LAU203	100	38[c]	29	7	17	37	41	15	6	32	83	18	81
	30	29	11	16	0	17	23	7	1	26	96	4	75
	10	3	6	2	0	9	17	0	0	17	73	1	62
LAU132	100	9	12	1	0	19	19	6	3	34	80	6	31
	30	3	7	2	0	5	10	1	2	16	32	3	18
	10	0	0	5	1	0	0	1	0	5	23	2	6
LAU145	100	15	24	4	1	39	40	5	9	29	80	6	30
	30	9	12	3	1	15	25	2	1	10	29	5	19
	10	3	6	0	0	4	6	0	0	10	16	3	7
LAU86	100	36	29	22	5	44	38	14	10	35	45	24	15
	30	17	15	9	5	20	26	6	0	24	23	10	4
	10	16	5	2	0	10	10	1	0	14	9	1	0
LAU50	100	21	26	7	5	18	20	3	5	19	26	6	20
	30	7	16	4	5	8	13	1	0	10	18	3	8
	10	7	7	0	4	0	4	1	0	3	12	0	0
LAU148	100	51	39	13	4	46	45	9	0	34	39	9	4
	30	19	8	5	4	20	26	1	2	19	27	9	3
	10	3	6	1	0	14	14	6	0	13	13	1	0
LAU161	100	24	22	6	1	33	31	3	1	25	38	4	23
	30	3	8	6	1	16	12	3	0	18	23	2	13
	10	2	0	3	0	9	7	2	0	5	11	3	4
LAU119	100	31	27	5	12	33	31	1	4	18	46	5	45
	30	7	13	1	1	17	23	3	4	13	39	4	25
	10	4	0	0	0	9	12	1	0	7	17	2	16
Clone 6	10	7	73	2	73								
	3	2	74	0	61								
	1	0	65	0	51								

[a]Lytic activity was assayed 7 days after the third restimulation.
[b]Lymphocyte to target cell ratio titration was performed for every assay.
[c]Numbers represent the percent specific lysis obtained for each target.
Me290 is a Melan-A and HLA-A*0201 positive melanoma cell line obtained from patient LAU203.
Me260 is a HLA-A*0201 negative melanoma cell line obtained from patient LAU149
Each number represents the geometric mean of duplicate cultures.
Bold face type indicate significant specific CTL.
When the differences in specific lysis obtained on T2 cells in presence or in absence of Melan-A 26-35 (1 μM) or Me290 and Me260 is equal or higher than 10%.
A patient is considered as responder when a significant specific lysis is detected in at least one of the cultures.
[d]Clone 6 is a Melan-A specific CTL clone derived from the TILN 289.